from a cell.

REMARKS

The Present Invention

The claimed invention is directed to antigen presenting vesicles free from their natural surroundings. The invention is also directed to methods of obtaining vesicles and of using them to stimulate T cells.

The Pending Claims

Prior to entry of the above amendments, claims 2-4, 6 and 9-13 are pending. Claims 2-4, 6 and 13 are directed to an antigen presenting vesicle. Claims 9, 11-12 are directed to a method for obtaining antigen presenting vesicles. Claim 10 is directed to a method for stimulating a T cell.

The Office Action

The restriction requirement is made final.

Claims 9-12 are withdrawn from further consideration.

The formal drawings submitted fail to comply with 37 CFR 1.84.

The application does not contain an abstract.

The specification is not in proper order.

Claims 2-4, 6 and 13 are rejected under 35 USC 112, first paragraph, enablement requirement.

Claims 2-4, 6 and 13 are rejected under 35 USC 102(e) over Melief USPN 5,731,160.

Claims 2-4, 6 and 13 are rejected under 35 USC 102(b) over Walden, *et al, Nature* (1985) 315:327.

Claims 2-4, 6 and 13 are rejected under 35 USC 102(b) over Harding and Gueze, *J Immunol* (1993) 151:3988.

Claims 2-4, 6 and 13 are rejected under 35 USC 102(b) over Amigorena, *et al, Nature*

(1994) 369:113.

Amendments

Applicants provide a substitute specification with text presented in the correct format.

Typographical errors in the original specification are corrected in the substitute specification, as identified above. In all instances, biotinilated is changed to biotinylated, lysosome is changed to lysosome, 70.000 is changed to 70,000.

Descriptions of the drawings on pages 8-10 of the original specification and specific references cited on pages 11-15 of the original specification are inserted on page 2 between lines 24 and 25.

Where numbers in the text referred to more detailed information on pages 11-15 of the original specification, that detailed information was inserted into the body of the text. For example, the text on page 12 referred to by reference number 16 on page 3, line 14, was inserted at page 3, line 14.

Applicants have amended Claims 4, 9 and 13.

Claim 4 was amended to recite only MHC class I proteins.

Claim 9 was amended to recite a MHC class I protein. Support is found on page 6, lines 3-6.

Claim 13 was amended to recite "obtainable from a cell" in the body of the claim.

Applicants believe that no new matter has been added by any of these amendments and respectfully request the Examiner to enter them.

Response

The Examiner's specific objections and rejections are reiterated below as small indented bold print, followed by Applicant's response in normal print.

Abstract.

This application does not contain an abstract of the disclosure as required by 37 CFR 1.72(b). An abstract on a separate sheet is required.

Applicants have provided an abstract on a separate sheet (*see* page 15 of substitute specification attached hereto).

Specification.

The following order or arrangement is preferred in framing the specification and, except for the title of the invention, each of the lettered items should be preceded by the headings indicated below.

- **(a) Title of the Invention**
- **(b) Cross-References to Related Applications (if any).**
- **(c) Statement as to rights to inventions made under Federall-sponsored research and development (if any).**
- **(d) Background of the invention.**
  - **1. Field of the Invention**
  - **2. Description of the Related Art including information disclosed under *37 C.F.R. §§ 1.97-199.***
- **(e) Summary of the Invention.**
- **(f) Brief Description of the Drawing.**
- **(g) Description of the Preferred Embodiment(s).**
- **(h) Claim(s).**
- **(i) Abstract of the Disclosure.**

Applicants attach hereto a substitute specification with a corrected order of arrangement.

35 U.S.C. § 112, first paragraph, enablement

Claims 2-4, 6 and 13 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

There is insufficient guidance in the instant specification and in the prior art for an antigen presenting vesicle free from its natural surroundings obtainable from an antigen presenting cell comprising a membrane and an MHC Class I protein or fragment thereof, as recited in claim 13, and its dependent claims 2-4 and 6, though the specification, is enabling for an antigen presenting vesicle free from its natural surroundings obtainable from an antigen presenting cell comprising a membrane and an MHC class II protein fragment thereof. As evidenced by Figure 1 in Delves et al., (Molecular Medicine Today, 3(2):55-60, 1997), it well known in the art that MHC Class I and Class II antigen presentation follow distinct, compartmentalized pathways, and that Class II bind antigens in endosomes or specialized loading compartments that are distinct from Class I molecules. Therefore, it is not clear from the instant specification that it is possible to isolate exosomes comprising class I (as opposed to class II) molecules from the supernatant of antigen presenting cells as taught by the specification.

Based upon the paucity of information contained within the instant specification in this regard, it would require an undue amount of experimentation on the part of one skilled in the art to use the claimed polypeptide for the asserted utilities.

In view of the quantity of experimentation necessary to use the claimed invention, the lack of

working examples, the unpredictability of the art, the lack of sufficient guidance in the specification, it would require an undue amount of experimentation on the part of one skilled in the art to use the claimed methods for the asserted utilities, and this is not sanctioned by the statute.

Applicants respectfully traverse the rejection of Claims 13, 2-4 and 6 as unpatentable under 35 USC 112, first paragraph, because the methods of differential centrifugation and sucrose gradients used to prepare subcellular fractions, including exosomes are standard, well-known techniques in the art and were known to those of ordinary skill in the art at the time of the priority date for the instant application.

Legal Standard

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosure in the specification coupled with information known in the art without undue experimentation. A patent need not teach, and preferably omits, what is well known in the art. MPEP 2164.01.

Methods of isolating exosomes are within the ordinary skill in the art

Applicants assert further that the techniques of differential centrifugation and sedimentation gradients were well known in the art at the time of the August 1995 priority date for the instant application. Furthermore, information regarding how to apply the techniques of differential centrifugation and/or sedimentation gradients to enrich for a particular desired subcellular fraction were available to those in the art. Furthermore, because such *methods* can be designed to selectively isolate any desired subcellular fraction, they are equally applicable to obtaining an antigen presenting vesicle free from its natural surroundings comprised of a membrane and a major histocompatibility complex MHC class I protein as to obtaining a vesicle comprising a class II protein. It is only necessary to determine from knowledge of the cell trafficking pathways for an MHC molecule which fraction(s) to isolate, and these pathways were known in the art. It was well known in the art at the time of application that MHC class I proteins are synthesized in the endoplasmic reticulum (ER) and transported

through the Golgi complex to the cell surface (*see* for example, page 3997, col. 1, of Harding and Geuze, a reference cited by the Examiner and Peters, *et al, Nature* (1991) 349:669 and Neefjes, *et al, Cell* (1990) 61:171, abstracts attached). Techniques for specifically obtaining ER or Golgi-enriched fractions from cell lysate were readily available in the public domain (*see,* for example, pages 113 and 114 of Amigorena, *et al, Nature* (1994) 369:113, a reference cited by the Examiner, and also Fath, *et al, J Cell Biol* (1993) 120:117; Linstedt, *et al, Mol Biol Cell* (1993) 4:679; Hashimoto, *et al, J Biol Chem* (1993) 268:25857; and Schmitz, *et al, Eur J Cell Biol* (1994) 63:77-83, abstracts attached).

The specification describes subcellular fractionation techniques applicable to both cell culture supernatants and to cell lysates

Applicants have described methods of differential centrifugation and isolation of subcellular fractions over sucrose gradients in the original specification (*see* page 8, line 23 through page 9, line 8 and page 11, line 36 through page 12, line 29 of the original specification) that are equally applicable to cell culture supernatants and cell lysates (*see* page 12, line 30 through page 13, line 13 of the original specification).

Applicants teach by example how to use an antigen presenting vesicle

The Examiner states that it would require an undue amount of experimentation to *use* the claimed *polypeptide* [sic] for the asserted utilities (emphasis added). Applicants assume the Examiner to intend the claimed antigen presenting vesicles. Applicants specifically teach how to use vesicles having MHC molecules to present antigen to T cells and to induce a specific T cell response on page 4, line 27 through page 5, line 8, and on page 10, lines 11-27 of the original specification. The basic use of antigen presenting vesicles carrying MHC class I or class II proteins is the same and assays to measure specific T cell responses were standard and well-known in the art at the time of application. One of skill in the art would know that when using vesicles with MHC class I molecules, induction of a specific T cell response is measured by employing a standard cytolytic T-lymphocyte assay (described in the Melief '160 patent)

rather than a proliferation assay. Because T-cell stimulation assays were standard to those of ordinary skill in the art, undue experimentation is not required. Applicants describe further uses of the antigen presenting vesicles on page 7, lines 3-23. The basic use of the antigen presenting vesicles in these applications is intrinsically the same, whether they carry MHC class I or MHC class II proteins.

Conclusion

One of ordinary skill in the art could readily combine the information available in the public domain regarding the subcellular trafficking of MHC class I proteins and subcellular fractionation techniques, with the established protocols outlined in the specification for differential and sucrose gradient centrifugation to obtain antigen presenting vesicles from a cell free from their natural surroundings comprised of a membrane and a MHC class I protein. Obtaining antigen presenting vesicles from a cell with MHC class I proteins would not involve undue experimentation, because information on the subcellular localization of MHC I proteins and on how to isolate particular desired subcellular fractions was readily available in the public domain. In view of all the foregoing reasons, the Examiner is respectfully requested to withdraw this rejection.

35 U.S.C. § 102(e), Melief USPN 5,751,160

Claims 13, 2, 3, 4 and 6 are rejected under 35 U.S.C. § 102(e) as being anticipated by Meleif et al. (U.S. Patent No. 5,731,160, 1998).

Melief et al. teach an antigen specific bilayer carrying vesicles from artificial lipid bilayer systems, as well as cells such as antigen presenting cell lines RMA-S, said vesicles incorporating MHC molecules that can be loaded with exogenous peptides (see entire article, including column 5, lines 47-63 and 20-26). Therefore, the referenced teachings anticipate the claimed references.

Applicants respectfully traverse the rejection of Claims 13, 2-4 and 6 as being anticipated by Melief USPN 5,751,160, because Melief does not teach or suggest antigen presenting vesicles.

Legal Standard

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. MPEP 2131.

The Melief '160 patent does not teach or suggest vesicles of any kind

The '160 patent is primarily concerned with antigen-processing-defective cell lines having empty MHC class I molecules with peptide. The main concern of the invention disclosed in the '160 patent is to induce a primary cytotoxic T-lymphocyte (CTL) response (abstract, col. 4, lines 60-64) using cells that are defective in endogenous antigen processing. In the passage on which this rejection is primarily based in col. 5, lines 48-63, it is stated that the invention of the '160 patent is "extended to all antigen presenting lipid bilayer carrying vehicles incorporating empty MHC molecules that can be loaded with exogenous peptides (col. 5, lines 53-55 of the original specification), but nowhere does the '160 patent teach or suggest antigen presenting vesicles obtainable from a cell. Therefore, the '160 patent can not properly anticipate the invention set forth in Claims 13, 2-4 and 6 because the '160 patent does not teach, suggest or mention anything pertaining to antigen presenting vesicles of any kind. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

35 U.S.C. § 102(b), Walden

> **Claims 13, 2, 3, 4 and 6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Walden et al. (Nature, 315:327-329, 1985). (C1 IDS)**
>
> **Walden et al. teach liposomes carrying a foreign protein antigen and MHC Class II molecules from membrane bound spleen cells which contain antigen presenting cells, and also that foreign antigens were attached to the lipid membranes that were capable of stimulating T cells in an antigen specific manner (see entire article especially page 327, column 2, last 2 paragraphs). Therefore, the referenced teachings anticipate the claimed references.**

Applicants respectfully traverse the rejection of Claims 13, 2-4 and 6 as being anticipated by Walden *et al*, because this reference does not teach or suggest (i) an antigen presenting vesicle comprising a membrane and a *MHC class I* protein, or (ii) an antigen presenting vesicle that is *obtainable from an antigen presenting cell.*

Walden does not teach or suggest vesicles with MHC class I molecules

The claims, as amended, are directed to an antigen presenting vesicle that comprises a membrane and a MHC class I protein. Walden does not teach or suggest anything about antigen presenting vesicles, synthetic or obtainable from a cell, that have MHC class I molecules. Walden does not even mention MHC class I proteins, and the Examiner has made it of record that antigen presenting vesicles comprising MHC class II proteins are a distinct invention from antigen presenting vesicles comprising MHC class I proteins (*see* paper 10, page 4, paragraph 5 and paper 13, page 2, paragraph 3). Since the two classes of antigen presenting vesicles are patentably distinct, the vesicles comprising class I proteins are novel over vesicles comprising class II proteins.

Walden describes synthetic vesicles that are not obtainable from a cell

Walden discloses a synthetic lipid vesicle with inserted, purified MHC class II molecules and protein antigen that is covalently linked to the lipid. Walden prepared liposomes from dipalmitoylphosphatidylethanolanine (DPPE), of which 10% were activated with N-succinimidyl 3(-2-pyridyldithio)propionate (SPDP) to covalently couple ovalbumin or bovine insulin. Furthermore, the lipid bilayer compositions taught by Walden are not obtainable from a cell, as applicants note in the original specification.

In view of the foregoing, Applicants respectfully assert that Walden does not anticipate antigen presenting vesicles obtainable from a cell that comprise a membrane and a MHC class I protein. The Examiner is respectfully requested to withdraw this rejection.

35 U.S.C. § 102(b), Harding

Claims 13, 2, 3, 4 and 6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Harding and Gueze (J. Immunology, 151:3988-3998, 1993). (C2 IDS)

Harding and Gueze teach the subcellular fractionation of murine peritoneal macrophages to produce fractions containing MHC Class II molecules (see entire article, especially page 3990, column 2, last 2 paragraphs), and that fractions containing lysozymes and light density membranes contained peptide-MHC-II complexes that were detected by T cells, (see entire article especially page 3992, column 1, last paragraph). Therefore, the referenced teachings anticipate the claimed references.

The open language of "comprising" is noted in claim 13.

Applicants respectfully traverse the rejection of Claims 13, 2-4 and 6 as being anticipated by Harding and Geuze, because this reference does not teach or suggest antigen presenting vesicles comprising a membrane and a MHC class I molecule. Harding and Geuze disclose vesicles from fractionated cells that have MHC class II molecules (Figure 3 on page 3993). Harding and Geuze are strictly concerned with the intracellular trafficking and intracellular antigen binding of MHC class II molecules. MHC class I molecules are not within the scope of the study presented in this manuscript, and Harding and Geuze do not teach or suggest anything about antigen presenting vesicles that comprise an MHC I molecule in this manuscript. As stated above, the Examiner has made of record that antigen presenting vesicles comprising MHC class II proteins are a distinct invention from antigen presenting vesicles comprising MHC class I proteins (*see* paper 10, page 4, paragraph 5 and paper 13, page 2, paragraph 3). Since the two classes of antigen presenting vesicles are patentably distinct, the vesicles comprising class I proteins are novel over vesicles comprising class II proteins. Therefore, Applicants respectfully assert that the disclosure of Harding and Geuze does not anticipate the claimed invention.

35 U.S.C. § 102(b), Amigorena

14. Claims 13, 2, 3, 4 and 6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Amigorena et al (Nature, 369:113-120, 1994). (C3 IDS)

Amigorena et al teach the subcellular fractionation of a B cell line to produce fractions containing membrane vesicles with MHC Class II molecules (see entire article, especially page 114, column 2, last paragraph), which contained processed peptide (see entire article, especially page 118, first paragraph of the Discussion Section). Therefore, the referenced teachings anticipate the claimed references. The open language of "comprising" is noted in claim 13.

Applicants respectfully traverse the rejection of Claims 13, 2-4 and 6 as being anticipated by Amigorena, because this reference also does not teach or suggest antigen presenting vesicles comprising a membrane and an MHC class I protein. Amigorena discloses an antigen presenting vesicle having MHC class II proteins derived from cells fractionated by free flow electrophoresis (*see* Figure 2 on page 115). Amigorena is strictly concerned with

characterizing the MHC class II-enriched intracellular compartments that they have identified. MHC class I proteins are outside the scope of the study presented in the Amigorena manuscript, and are not even mentioned. There is no disclosure in Amigorena to teach or suggest an antigen presenting vesicle comprised of a membrane and an MHC class I molecule. As stated above, the Examiner has made of record that antigen presenting vesicles comprising MHC class II proteins are a distinct invention from antigen presenting vesicles comprising MHC class I proteins (*see* paper 10, page 4, paragraph 5 and paper 13, page 2, paragraph 3). Therefore, Applicants respectfully assert that this reference does not properly anticipate the claimed invention. Since the two classes of antigen presenting vesicles are patentably distinct, the vesicles comprising class I proteins are novel over vesicles comprising class II proteins. The Examiner is respectfully requested to withdraw this rejection.

CONCLUSION

In view of the above amendment and remarks, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 328-4400.

Respectfully submitted,

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Jennifer L. Wahlsten, Ph.D.
Reg. No. 46,226

Rae-Venter Law Group, P.C.
P. O. Box 60039
Palo Alto, CA 94306
Telephone: (650) 328-4400
Facsimile: (650) 328-4477

BRV/JLW